

Docket No.: NEB-150

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: Evans EXAMINER: W. Moore

APPLICATION NO.: 09/786,009 GROUP: 1652

FILED: April 17, 2001

FOR: Intein-Mediated Protein Ligation

The Honorable Commissioner of  
Patents and Trademarks  
Washington, DC 20231

**PRELIMINARY AMENDMENT OF THE CLAIMS**

Sir:

Please cancel claims 1-6 and 8-11, amend claim 7 and add new claims 12- 27.

7. A cyclic protein produced by fusing an expressed protein with a peptide, using a method comprising:

- (a) generating at least one C-terminal thioester tagged protein;
- (b) generating at least one target peptide having a specified N-terminal; and
- (c) ligating the target peptide to the target protein.

12. A method for obtaining an expressed protein with a C-terminal thioester, comprising:

- (a) obtaining the expressed precursor protein, the precursor having an intein, an intein derivative or mutants thereof; and
- (b) reacting the precursor protein with a thiol reagent so as (i) to remove the intein and (ii) to obtain the expressed protein with the C-terminal thioester.